Appl. No. 10/523,459 Amdt. dated November 6, 2007 Reply to Office Action of August 9, 2007

Amendments to the Specification:

Please replace the paragraph beginning at page 7, line 23, with the following:

--FIGURE 3. MS/MS analysis of the PEB3 tryptic glycopeptide. a) Product ion spectrum of the doubly protonated glycopeptide ion at m/z 1057.9. The fragment ions originating from the sequential loss of oligosaccharide residues are indicated in the spectrum. The peptide sequence (SEQ ID NO:1) is shown in the inset. b) Second generation product ion spectrum of the glycopeptide fragment (SEQ ID NO:2) ion at m/z 937.4. The glycopeptide was fragmented by front end collision induced dissociation (orifice voltage = 100 V) as it entered the mass spectrometer. The observation of the b₃+228 fragment ion at m/z 605.3 confirmed that the oligosaccharide is *N*-linked.--

Please replace the paragraph beginning at page 15, line 23, with the following:

--Construction and characterization of pglB mutant: For construction of the pglB mutant, genes Cj1121c to Cj1126c were PCR amplified from *C. jejuni* NCTC 11168 using the primers: Cj1121cF (5'-ACTCACTATTGCCATTAAGATAAGC-3'; SEQ ID NO:3) and Cj1126cR (5'-AAAACCCTTATTTAGTTTTGCTTTGC-3'; SEQ ID NO:4). The PCR product was polished with *Pfu* polymerase and then ligated into pPCR-Script Amp (Stratagene) according to the manufacturer's instructions. The ligation mixture was electroporated into electrocompetent *E. coli* DH10B and selected for on LB S-gal agar (Sigma-Aldrich) with ampicillin. A blunt-ended kanamycin resistance cassette from pILL600 (37) was inserted into the filled-in *Xba*I restriction site of *pglB*, generating pEAp26. The orientation of the cassette was determined by sequencing with the ckanB primer (5'-CCTGGGTTTCAAGCATTAG-3'; SEQ ID NO:10). DNA was sequenced using terminator chemistry and AmpliTaq cycle sequencing kits (Applied Biosystems) and analysed on an Applied Biosystems 373 DNA sequencer. The

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mutated plasmid DNA was used for electroporation into *C. jejuni* NCTC 11168 (32) and the kanamycin-resistant transformants were characterized by PCR to confirm that the incoming plasmid DNA had integrated by a double cross-over event.--

Please replace the paragraph beginning at page 18, line 19, with the following:

-- The mass spectrum and the reconstructed molecular mass profile for fraction # 31 are presented in Figure 2 a) and b). Three peaks were observed in the reconstructed mass profile. The peaks at 25,454 Da and 28,376 Da correspond well with the expected molecular masses of PEB3 (25,453 Da, Cj0289c) and PEB4 (28,377 Da, Cj0596) respectively, without signal peptides. To identify the protein of mass 26,861 Da, CapLC-MS/MS analysis was carried out on the tryptic digest of this fraction. All but one of the peptides identified could be assigned to PEB3 or PEB4, in accord with the N-terminal sequence data. MS/MS analysis of the unidentified ion (Fig. 3a) clearly identifies it as a glycopeptide. A fragmentation series composed of sequential losses of HexNAc (203 Da) and a single Hex (162 Da) can be observed in this spectrum. The tryptic peptide was identified as ⁶⁸DFNVSK⁷³ (SEQ ID NO:2) from PEB3. The residue mass of the oligosaccharide portion of this glycopeptide is 1406 Da, which corresponds well with the difference in the molecular weights of PEB3 and the unknown protein peak observed in Figure 2b. Therefore, it appeared that approximately 50% of the PEB3 protein in this fraction was modified with a single oligosaccharide composed of 5 HexNAcs, 1 Hex and an unusual sugar with a residue mass of 228 Da. Moreover, the MS/MS spectrum indicated that the oligosaccharide was linked to the peptide via the 228 Da sugar moiety.--

Please replace the paragraph beginning at page 19, line 26, with the following:

--Putative glycoproteins were purified by SBA affinity chromatography from the glycine extracts of 40 g wet weight of cells. The yield of putative glycoproteins was 5 mg as estimated by UV absorbance at 280 nm. The GalNAc eluant was subjected to 1D- and 2D-

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PAGE (Fig. 4) and to ensure that the proteins purified in this manner possessed lectin binding properties, rather than non-specific binding characteristics, western blotting with an SBA/alkaline phosphatase conjugate was also carried out. Approximately 13 protein species were visualized following 1D SDS-PAGE but this number increased substantially when the product was analyzed by 2D-PAGE. The proteins in individual bands from 1D SDS-PAGE and spots from 2D-PAGE were identified by mass fingerprinting and database searching (Table I). Among the identified proteins are PEB3 (Ci0829c) and CgpA (Ci1670c) previously identified by Linton et al. (8). The vertical pattern of spots with identical pIs displayed by Cj1670c, and other proteins, likely indicates varying degrees of glycosylation since examination of their predicted amino acid sequences, derived from the whole genome sequence of C. jejuni NCTC 11168 (6), revealed the presence of multiple potential N-linked glycosylation sites containing the sequon Asn-Xaa-Ser/Thr (Table I). In fact, MS/MS analysis of the Ci1670c-containing in-gel digest extracts indicated that 3 of its 6 N-linkage sites are occupied to varying extents (three Cj1670c glycopeptides were detected by capLC-MS/MS: ⁷TDQNITLVAPPEFQKEEVK²⁵ (SEQ ID NO:5), ⁷⁷VLDVSVTIPEKNSSK⁹¹ (SEQ ID NO:6) and ⁹²QESNSTANVEIPLQVAK¹⁰⁸ (SEQ ID NO:7). A single glycopeptide was also observed for Ci0114 (71LSQVEENNQNIENNFTSEIQK91; SEQ ID NO:8) and for Cj0200c (¹DSLKLEGTIAQIYDNNK¹⁷; SEQ ID NO:9). Furthermore, the mass and composition of the glycan component of all these glycopeptides appears to be identical to that observed for PEB3.--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1-3, at the end of the application.